Photoswitchable nanoparticles for in vivo cancer chemotherapy

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Photoswitchable nanoparticles for in vivo cancer chemotherapy

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There are many obstacles to effective cancer chemotherapy, including drug penetration and accumulation in tumors and drug systemic toxicity. The penetration of therapies into tumors is limited by the dense tumor matrix and by compression of the tumor vasculature. We have developed spiropyran-based nanoparticles that shrink from 103 to 49 nm upon irradiation at 365 nm. That shrinkage enhanced tissue penetration and drug release. Irradiation of s.c. HT-1080 tumors in nude mice administered i.v. docetaxel-containing nanoparticles was more effective treatment than free docetaxel or encapsulated docetaxel without irradiation. Irradiation at the tumor site also resulted in less systemic toxicity than if the nanoparticles were irradiated before injection, presumably because of less systemically distributed free drug. The enhanced efficacy of nanoparticles in irradiated tumors may have been related to the observed enhanced tumor penetration by nanoparticles and decompression of tumor blood vessels, which may also increase nanoparticle delivery into tumors.

Results

Light-Triggered Size Change and Dtxl Release. We have previously shown that hybrid NPs (NP\textsubscript{HC}s; \textit{SI Appendix, Fig. S1}) comprising spiropyran (SP) and lipid-polyethylene glycol (PEG) undergo a reversible volume change from 150 nm to 40 nm upon phototrigging (22). UV light (365 nm) induces hydrophobic SP to switch to zwitterionic merocyanine (MC; \textit{SI Appendix, Fig. S1A}) (23), which alters the NP\textsubscript{HC}'s physical assembly properties with an accompanying decrease in volume. Because MC is less stable than SP, MC NP\textsubscript{HC}s spontaneously revert to SP NP\textsubscript{HC} in darkness or under visible light, with an increase in NP size. Such reversible photoswitching of SP NP\textsubscript{HC} can enable repeated dosing from a single administration, and illumination enhances tissue penetration ex vivo.

Drug release in the absence of light triggering (i.e., in the off state) was minimized by increasing the hydrophobic interaction among lipids and SP by the introduction of cholesterol, which interacts strongly with phospholipids in liposomes to reduce bilayer permeability (24). NP\textsubscript{HC}s (NP\textsubscript{HC} with cholesterol) were obtained by sonication of 1 wt% cholesterol with SP and lipid-PEG, and had an average hydrodynamic diameter of 103.5 ± 4.1 nm and a polydispersity of 0.03 ± 0.01. After UV illumination (1 W/cm\textsuperscript{2}, 35 s, ∼100% conversion to MC), MC NP\textsubscript{HC} size decreased to 49.2 ± 3.3 nm (polydispersity of 0.05 ± 0.02, n = 5; Fig. 1C). NP\textsubscript{HC}s with adjustable loadings of Dtxl (Dtxl/SP NP\textsubscript{HC}s) up to 12.6 wt% and low polydispersities were readily obtained with sizes and photoswitching capabilities similar to those of NP\textsubscript{HC} (\textit{SI Appendix, Table S1}).

In the absence of UV light triggering, Dtxl was released slowly from SP NP\textsubscript{HC} in PBS (Fig. 1B). Upon UV irradiation (10 s), NP\textsubscript{HC}s (Dtxl wt% = 9.4%) released 16.1% of the loaded Dtxl within 1 h as determined by HPLC, whereas less than 5% was released in the same period without UV irradiation (Fig. 1B). UV light triggering (10 s) conducted every 12 h for three cycles caused repeated increases in release at each event (n = 5; Fig. 1B).

Significance

The importance of this research is in the demonstration of the effectiveness and improved safety of a nanoparticulate chemotherapeutic formulation that can be phototriggered to shrink in size at the tumor site. That shrinkage enhanced nanoparticle penetration into tumors and also triggered local drug release. The result was increased efficacy and reduced systemic toxicity. The phototriggered formulation also relieved the compression of tumor blood vessels, which is a recognized barrier to nanoparticle accumulation in tumors.

Author contributions: R.T. and D.S.K. designed research; R.T. and H.H.C. performed research; R.T. and D.S.K. analyzed data; and R.T. and D.S.K. wrote the paper.

The authors declare no conflict of interest.

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The importance of this research is in the demonstration of the effectiveness and improved safety of a nanoparticulate chemotherapeutic formulation that can be phototriggered to shrink in size at the tumor site. That shrinkage enhanced nanoparticle penetration into tumors and also triggered local drug release. The result was increased efficacy and reduced systemic toxicity. The phototriggered formulation also relieved the compression of tumor blood vessels, which is a recognized barrier to nanoparticle accumulation in tumors.

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Light-triggered drug release increased the cytotoxicity of Dtxl/SP NP\textsubscript{HC} in HT-1080 cells. Cells were incubated with Dtxl/SP NP\textsubscript{HC} for 4 h, then washed and incubated in media without NP\textsubscript{HC}, irradiated (10 s), and further incubated for a total of 24 h. [We have previously found that SP NP\textsubscript{HT} could be internalized by HeLa cells within 2 h (22).] Cell viability was then measured with an (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (25). Light-triggered Dtxl/SP NP\textsubscript{HC}s were more cytotoxic than Dtxl/SP NP\textsubscript{HC}s without light triggering \((n = 6, P < 0.005; \text{Fig. 1C}),\) presumably due to UV-triggered rapid intracellular release of Dtxl (22). SP NP\textsubscript{HC}s themselves (no drug), with or without UV irradiation (10 s), did not cause significant cytotoxicity in HT-1080 cells except at extremely high concentrations (SI Appendix, Fig. S2).

We verified that the cholesterol-modified SP NP\textsubscript{HC}s retained the light-triggered enhancement of diffusion that we had reported for NP\textsubscript{HT} (22) by measuring diffusion through a well-established one-dimensional collagen gel model (26) with the same collagen concentration as in the matrix of 6–8-mm-diameter HT-1080 tumors \([3.72 \pm 0.63 \text{ mg/g tumor} (n = 5)\) determined by a colorimetric hydroxyproline assay]. SP NP\textsubscript{HC}s (1 mg/mL) containing the dye Cy5 (1 wt%) penetrated 6.7 ± 0.2 mm within 8 h at 37 °C without UV triggering; when triggered by UV for 10 s, they penetrated 10.5 ± 0.3 mm (56% further; \(n = 4, P < 0.005\)). [The mechanical properties of collagen are essentially unchanged by 1 h irradiation at 254 nm UV light at 15.8 J/cm\(^2\).]

**UV Irradiation ex Vivo.** We investigated the irradiation time required to trigger photoswitchable SP NP\textsubscript{HC}s through the skin. The absorbance at 365 nm of mouse skin \([from 6 to 8 wk nu/nu (nude) mice] was 0.758 ± 0.066 (n = 4)—that is, ~17% of light at 365 nm was transmitted (SI Appendix, Fig. S3A, black curve). Light absorption of skin above s.c. HT-1080 tumors was similar (SI Appendix, Fig. S3A, red curve). SP NP\textsubscript{HC}s exhibited rapid photoswitching kinetics (half-time 14.8 s, same as described for NP\textsubscript{HT}) (22); through skin, the half-time was 25.9 s at a UV intensity of 1 W/cm\(^2\) (SI Appendix, Fig. S3B). The half-time of the conversion from MC NP\textsubscript{HC} to SP NP\textsubscript{HC} is 2.741 ± 27 s in vitro. This suggests that the triggered NPs would remain as MC NP\textsubscript{HC}s (i.e., reduced in size) for a few hours, during which they would penetrate tumors.

**Intratumoral Injection of NP\textsubscript{HC}s.** As a preliminary to studies of i.v. administered SP NP\textsubscript{HC}s, we examined their intratumoral distribution and effectiveness when delivered by direct injection at the tumor site. [Perforce this entailed NP deposition at the periphery of the tumors due to the difficulty of injecting into the high pressure deep in the tumor (28).] SP NP\textsubscript{HC}s containing the fluorescent dye Cy5 (1 mg/kg) were injected into s.c. HT-1080 tumors \((diameters \sim 6–8 \text{ mm)} with or without immediately subsequent light triggering (15 s, 1 W/cm\(^2\)). Frozen sections of tumors collected 12 h postinjection revealed that the fluorescence of Cy5/SP NP\textsubscript{HC}s was restricted to the injection site in the absence of light triggering, whereas light-triggered (365 nm, 15 s) NP\textsubscript{HC}s were more broadly distributed \((Fig. 2A and B),\) as quantified by percentage of tumor area positive for Cy5 fluorescence \((2C; n = 4, P < 0.001\). It is unlikely that the brief irradiation itself \((15 \text{ J/cm}^2) altered the tumor vasculature; for UV light over 320 nm, the dose to cause erythema in mice skin is \(\sim 3,000 \text{ J/cm}^2\) (29–31). Therefore, the increased distribution of fluorescence after irradiation was likely due to enhanced diffusive movement of NP\textsubscript{HC}s.

We evaluated the efficacy of Dtxl/SP NP\textsubscript{HC} in s.c. implanted HT-1080 tumors in nu/nu mice, once those tumors reached ca. 100 mm\(^3\) in size. The following treatments were administered as a single intratumoral injection \((n = 5)\): \(i\) PBS, \(ii\) PBS with irradiation (365 nm, 10 s, 1 W/cm\(^2\)), \(iii\) Dtxl/SP NP\textsubscript{HC} (Dtxl 10 mg/kg), and \(iv\) Dtxl/SP NP\textsubscript{HC} with irradiation (10 s, 1 W/cm\(^2\)) (Fig. 2C and D). Single intratumoral administrations of Dtxl/SP NP\textsubscript{HC}s were efficacious in tumor reduction, with and without light triggering \((Fig. 2D).\) Of note, two of five mice in the Dtxl/SP
NP\textsubscript{HC} group had to be euthanized per animal protocol, as their tumor volumes exceeded 500 mm\textsuperscript{3} (~1 cm diameter) on days 18 and 29 (SI Appendix, Fig. S4F). In contrast, all mice treated with Dtxl/SP NP\textsubscript{HC}S and irradiation showed significant tumor reduction. In the groups administered PBS only (without and with light triggering), the tumors in all animals grew within 2 wk to the point where euthanasia was indicated. Animals treated with Dtxl/SP NP\textsubscript{HC} did not show appreciable changes in body weight over 15 d, suggesting no severe systemic toxicity (SI Appendix, Fig. S4B).

**Toxicity of Systemically Administered NP\textsubscript{HC}S.** Animals were given a single i.v. bolus of SP NP\textsubscript{HC}S or PBS in mice with 4–5-mm-diameter s.c. tumors at a high dosage of 400 mg/kg. Particles were drug free or contained Dtxl (40 mg/kg; dosage based on reported maximum (olerated dosage) (32). The tumor sites were irradiated (1 W/cm\textsuperscript{2}) for 20 s) or not in animals receiving SP NP\textsubscript{HC}S containing Dtxl. Mice remained healthy during an observation period of 2 wk, without behavior changes or severe weight loss (<10\%). Blood tests suggested that the hepatic function of the mice was normal (for alkaline phosphatase and alanine transferase, $P > 0.1$ for the comparison between mice treated with PBS and Dtxl/SP NP\textsubscript{HC}S with irradiation; SI Appendix, Fig. S5). Red blood cell counts and characteristics were also not affected by the large dose of SP NP\textsubscript{HC}S ($P > 0.2$ between mice treated with PBS and Dtxl/SP NP\textsubscript{HC}S with irradiation; SI Appendix, Fig. S6). White blood cell counts were also not affected ($P > 0.2$ between mice treated with PBS and Dtxl/SP NP\textsubscript{HC}S with irradiation) and platelet counts were not depressed (SI Appendix, Fig. S7). Hematoxylin-eosin-stained histological sections of organs harvested 72 h postinjection were normal (SI Appendix, Fig. S8).

**Photostwitching Enhances Efficacy of Dtxl NP\textsubscript{HC}S.** We evaluated the efficacy of i.v.-injected Dtxl/SP NP\textsubscript{HC} in s.c. HT-1080 tumors (Fig. 3 A and B; SI Appendix, Table S2). After tumors reached 100–150 mm\textsuperscript{3}, mice were treated with the following regimens ($n = 5$): (i) PBS, (ii) Dtxl, (iii) Dtxl/SP NP\textsubscript{HC}, (iv) Dtxl/SP NP\textsubscript{HC} converted to Dtxl/MC NP\textsubscript{HC} by irradiation just before injection (“preirradiation”), or (v) Dtxl/SP NP\textsubscript{HC} with irradiation (20 s, 1 W/cm\textsuperscript{2}) onto the tumor site 30 min postinjection (“post-irradiation”); see SI Appendix, Methods S6 for the rationales for irradiation times). The Dtxl dosing was 40 mg/kg. Mice treated with free Dtxl experienced severe weight loss (over 20\%) within 4 d; significant body weight loss at day 6 was also seen in the group treated with Dtxl/MC NP\textsubscript{HC} (preirradiation group, SI Appendix, Fig. S4C), presumably due to the considerable free Dtxl released from MC NP\textsubscript{HC} upon irradiation (16.1\% Dtxl was released in vitro within 1 h; Fig. 1B).

In the group treated with PBS, tumor volume rapidly exceeded 500 mm\textsuperscript{3} (ca. tumor diameter over 1 cm) with a median tumor volume doubling time of 2.98 d. In contrast, in mice dosed with Dtxl/SP NP\textsubscript{HC} the median tumor volume doubling time increased to 7.55 d (SI Appendix, Table S2), and all tumors reached 500 mm\textsuperscript{3} (ca., tumor diameter over 1 cm) within 20 d. Irradiation of Dtxl/SP NP\textsubscript{HC} at the tumor site (postirradiation) greatly improved efficacy: three of five mice survived over 100 d, with two mice having complete tumor resolution (comparison of survival curves for Dtxl/SP NP\textsubscript{HC} groups with and without irradiation, by log-rank test, $P = 0.004$).

Triggering at the tumor site affected the efficacy and toxicity of Dtxl/SP NP\textsubscript{HC}. The mouse body weights in the preirradiation group were lower than in postirradiated animals at day 12 ($P = 0.04$; SI Appendix, Fig. S4C). All animals in the preirradiation group were euthanized by day 75 due to tumor growth over 500 mm\textsuperscript{3} (vs. three survivors to 100 d in postirradiated animals). The median tumor volume on day 53 in the preirradiation group was 163.8 mm\textsuperscript{3} (two animals) versus 28.8 mm\textsuperscript{3} in postirradiated animals (three animals). However, the difference between survival curves of preirradiation and postirradiation groups was not statistically significant ($P = 0.093$ by log-rank test). The differences between the in vivo results in preirradiation and postirradiation groups likely reflected the fact that in the preirradiation group, more free drug was released systemically, whereas in postirradiated animals, more Dtxl was released in the tumor. Histological studies (SI Appendix, Fig. S9) showed that 96 h postinjection live tumor cells in the light-triggered Dtxl/SP NP\textsubscript{HC} group were significantly decreased (tumor cell density decreased...
by 51.5% compared with the group treated with PBS, \( P < 0.005 \), whereas the groups treated with the same NP\(_{1HCS}\) without or free Dtxl had decreases in cell density of 23.5% and 8.7%, respectively (\( P = 0.05 \) and 0.30 in comparison with the group treated with PBS, respectively).

**NP Pharmacokinetics and Biodistribution.** Because particle size has a marked impact on pharmacokinetics and biodistribution (33–36), we investigated whether light triggering at the tumor site would affect those parameters for Dtxl delivered by SP NP\(_{1HCS}\). The Dtxl concentration in blood plasma over time was fitted to a two-compartmental PK model. The mean elimination half-time (\( t_{1/2} \)) of Dtxl/SP NP\(_{1HCS}\), with or without light triggering (376 and 213 min, respectively; Fig. 5C and SI Appendix, Table S3), was significantly greater than that of free Dtxl (14 min, \( P < 0.005 \)). The areas under the curves (AUCs) for Dtxl/SP NP\(_{1HCS}\) without or with light triggering were both ~sixfold higher than the corresponding AUC for free Dtxl (SI Appendix, Table S3). Irradiation did not change the particle clearance rate (\( P = 0.27 \)).

Biodistribution was studied by measuring the mean tissue concentration of Dtxl by HPLC in various organs 24 h after injection (Fig. 3D). The majority of Dtxl/SP NP\(_{1HCS}\) accumulated in the liver and spleen, as is the case with many nanoparticulate systems (37, 38). The mean tumor uptake of Dtxl in light-triggered Dtxl/SP NP\(_{1HCS}\) was 1.7-fold and 6.6-fold higher than those of nontriggered NP\(_{1HCS}\) and free Dtxl, respectively (\( P < 0.005 \) for both).

**Tumor Penetration by Phototriggered NPs.** NP distribution within tumor tissues was investigated after injecting Cy5/SP NP\(_{1HCS}\) i.v. and irradiating the tumor sites 30 min after administration (20 s, 1 W/cm\(^2\)). Tumor tissues were collected 24 h postinjection for immunofluorescent imaging of frozen sections. In the absence of irradiation, Cy5 fluorescence intensity remained largely localized to tumor vessels (demarcated by endothelial cells stained by anti-CD31), as indicated by the yellow color (colocalization of intratumoral vessels and Cy5/SP NP\(_{1HCS}\)) in Fig. 4A, whereas Cy5 fluorescence was broadly distributed throughout the tumor tissue in irradiated mice (Fig. 4B). Quantitative analysis confirmed that the distribution of Cy5/SP NP\(_{1HCS}\) was more diffuse throughout the tumor in irradiated animals, as seen in the increased percentage of tumor area positive for Cy5 fluorescence and the increased distance of extravasated NP\(_{1HCS}\) from blood vessels (SI Appendix, Fig. S10).

**Effect of Light-Triggered Intratumoral Drug Release and Enhanced Penetration on the Tumor Vasculature.** We hypothesized that the increased tumor killing by photoswitching Dtxl/SP NP\(_{1HCS}\) was due to tumor cell apoptosis and tumor vessel decompression resulting from light-triggered Dtxl release from NP\(_{1HCS}\). The vascular decompression would enhance NP delivery into tumors. Studies have shown that systemic administration of taxanes (paclitaxel or Dtxl) can decompress intratumoral collapsed blood vessels and increase tumor blood flow without a change in vessels numbers (39), improving the delivery of therapeutics and improving tumor response (40–44). Treatment with Dtxl/SP NP\(_{1HCS}\) irradiated at the tumor site increased apoptosis (assayed by TUNEL staining) and decreased tumor cell proliferation (assayed by ki67 staining) in HT-1080 tumors 24 h after injection, compared with other treatment groups (SI Appendix, Fig. S11A). Tumor vessel density was not affected in any treatment group (SI Appendix, Fig. S11B). To assess the effect of treatments on blood vessel size, we measured vessel diameters in the periphery and interior of HT-1080 tumors (Fig. 4C–E; SI Appendix, Fig. S12). Vascular diameters were significantly increased (\( P < 0.001 \)) in the periphery of tumors treated with Dtxl/SP NP\(_{1HCS}\), with or without irradiation, whereas those diameters did not change significantly in the untreated and free Dtxl groups (Fig. 4C–E; SI Appendix, Fig. S12). Importantly, vascular diameters were increased in the tumor interiors, and vessels had open lumens, in animals treated with Dtxl/SP NP\(_{1HCS}\) with UV light irradiation (Fig. 4E, SI Appendix, Fig. S12), compared with those treated with free Dtxl or nontriggered particles (Fig. 4C and D; SI Appendix, Fig. S12). In the single animal treated with light-triggered Dtxl/SP NP\(_{1HCS}\) that was examined at 96 h, most tumor blood vessels had lumens over 15 \( \mu m \) (SI Appendix, Fig. S13).

To determine whether the increased vessel caliber affected blood perfusion, we identified perfused tumor vessels by staining with i.v. injected fluorescein–lectin (45–47). We calculated the percentage of all vessels (stained by anti-CD31) that were perfused (stained by fluorescein–lectin). At 24 and 48 h post-injection, Dtxl/SP NP\(_{1HCS}\) with light irradiation significantly increased the percentage of perfused blood vessels, compared with groups treated with Dtxl/SP NP\(_{1HCS}\) or free Dtxl (both \( P < 0.01 \); SI Appendix, Fig. S11C). These changes in the vasculature, along with the enhanced perfusion, suggest a decrease in intratumoral pressure (15, 20, 39, 48–51). The increased fraction of perfused blood vessels due to the decompression of blood vessels was associated with enhanced intratumoral accumulation of NP\(_{1HCS}\); Dtxl delivered by Dtxl/SP NP\(_{1HCS}\) with irradiation led to intratumoral accumulation of 6% of the injected dose per gram.
Effects of light triggering of Dtxl/SP NP_{PC} in the tumor vasculature. The dense collagen matrix (green lines) and compressed vessels prevent unshrunken NP_{PC} from delivering drugs within the tumor (gray region). Tumor irradiation shrinks NP_{PC} (purple spheres), which enhances their penetration through the collagen matrix throughout the tumor. The triggered release of Dtxl (the cyan glow surrounding NP_{PC}) kills tumor cells, which leads to dilation of compressed intratumoral blood vessels. The decompression of vessels facilitates the transport of NP_{PC} into the tumor interior.

Discussion

Light-triggered Dtxl/SP NP_{PC} induced tumor cell death (SI Appendix, Fig. S11A) and increased intratumoral vessel diameters and tumor perfusion (Fig. 4E; SI Appendix, Fig. S11C). These effects, along with the particles' light-triggered ability to penetrate collagen matrices, enhanced the accumulation of NP_{PC} through tumor tissues (Fig. 4F) and inhibited tumor growth (Fig. 3A), as schematized in Fig. 5. UV-triggered size change and drug release both affected drug accumulation in the tumor (the %I.D./g), by different mechanisms. Irradiation-induced size change enhanced particle penetration and hence the %I.D./g (Fig. 5D), but did not affect the tissue structure itself in the absence of drug release (SI Appendix, Fig. S11B). UV-triggered drug release led to vessel decompression to a greater extent than did nontriggered drug release (Fig. 4E). This vessel decompression, which was established by 24 h at the latest (Fig. 4C–E), perhaps further facilitated the penetration of drug-containing particles (Fig. 4F).

It is possible that the portion of the effect of the triggered Dtxl/SP NP_{PC} that is attributable to tumor penetration could be achieved by injecting NPs with the smaller size of the posttriggered MC NP_{PC}. However, those particles would not provide the beneficial effect of the triggered drug release within the tumor (Fig. 4E). A possible solution that would provide both enhanced penetration and triggered release would be to create triggerable particles that are formulated at the smaller size. A potential difficulty in that approach, however, is that most triggerable particles of that (or any) size (52, 53) tend to be depleted or destroyed by the single triggering event. Consequently, they would not be available after triggering to penetrate deeper into tissues and/or provide sustained drug release in situ—as would be the case with the formulation presented here. Moreover, this formulation has the potential for repeated triggering (Fig. 1B), although we did not study the antitumor effects of that capability in this report.

The wavelength used for triggering is of obvious importance. The wavelength at which SP is triggered could not be readily shifted to the near-infrared range as the covalent bond in SP requires a high-energy photon to break (54), and near-infrared light is relatively low in energy (E = hν). However, the photo-switching of SP NP_{PC} could potentially be triggered at depths up to centimeters by using near-infrared lasers (55) [e.g., by two-photon technology with wavelength ~720 nm (56–59)]. The use of near-infrared light would allow deeper tissue penetration, including through soft tissues, bone, and intact skull (53, 55, 60, 61). UV light could potentially also be used deep within the body by use of fiber optics and endoscopy (60–62).

The safety of the wavelength used also is important. UV light is divided into UVA (320–400 nm) and UVB (280–320 nm). UVA light has been used clinically to treat various diseases, including some skin conditions (e.g., psoriasis, vitiligo, atopic dermatitis) (63–65) and corneal collagen cross-linking (66, 67). Brief irradiation with 365 nm light at low energy is not considered a risk for skin cancer (68, 69). It bears mentioning that the treatment of cancers routinely involves forms of radiation that are potentially much more harmful (i.e., radiation oncology).

Conclusion

We have demonstrated the in vivo efficacy of photoswitchable NP_{PC} in a s.c. implanted tumor model. There have been a small number of strategies to enhance tumor penetration (21, 33–36, 48, 70–75), including applying external energy (e.g., heat) to dilate vessels (76, 77), using tissue-penetration peptides (78–80), or degrading the stromal matrix by applying proteases (e.g., collagenase and relaxin) (81–84). Improved tumor penetration by nanotherapeutics can also be achieved by co-administration of agents that enhance tumor perfusion by decreasing tumor interstitial fluid pressure (e.g., by inhibitors of TGF-β) (85, 86). Light-triggering of Dtxl/SP NP_{PC} increased NP diffusion through the tumor collagen matrix and induced release of Dtxl, which opened compressed intratumoral vessels by dilation of compressed intratumoral blood vessels. The decompression of vessels facilitates the transport of NP_{PC} into the tumor interior.
Tong et al.


